QuantiGlo®

Human IL-1β/IL-1F2 Immunoassay

Catalog Number QLB00B

For the quantitative determination of human interleukin 1 beta (IL-1β) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTRODUCTION

The Interleukin 1 (IL-1) family of proteins consists of the classic members IL-1α, IL-1β, and IL-1ra, plus IL-18, IL-33, and IL-1F5-F10. IL-1α and IL-1β bind to the same cell surface receptors and share biological functions (1). IL-1 is typically not produced by unstimulated cells of healthy individuals with the exception of skin keratinocytes, some epithelial cells, and certain cells of the central nervous system. However, in response to inflammatory agents, infections, or microbial endotoxins, a dramatic increase in the production of IL-1 by macrophages and various other cell types is seen. IL-1β plays a central role in immune and inflammatory responses, bone remodeling, fever, carbohydrate metabolism, and GH/IGF-I physiology. Inappropriate or prolonged production of IL-1 has been implicated in a variety of pathological conditions including sepsis, rheumatoid arthritis, inflammatory bowel disease, acute and chronic myelogenous leukemia, insulin-dependent diabetes mellitus, atherosclerosis, neuronal injury, and aging-related diseases (2-5).

IL-1α and IL-1β are structurally related polypeptides that show approximately 25% homology at the amino acid level. Both are synthesized as 31 kDa precursors that are subsequently cleaved into mature proteins of approximately 17.5 kDa (6, 7). Cleavage of the IL-1β precursor by Caspase-1/ICE is a key step in the inflammatory response (2, 8). Neither IL-1α nor IL-1β contains a typical hydrophobic signal peptide (9-11), but evidence suggests that these factors can be secreted by non-classical pathways (12, 13). A portion of unprocessed IL-1α can be presented on the cell membrane and may retain biological activity (14). The precursor form of IL-1β, unlike the IL-1α precursor, shows little or no biological activity in comparison to the processed form (13, 15). Both unprocessed and mature forms of IL-1β are exported from the cell.

IL-1α and IL-1β exert their effects through immunoglobulin superfamily receptors that additionally bind IL-1ra. The 80 kDa transmembrane type I receptor (IL-1 RI) is expressed on T cells, fibroblasts, keratinocytes, endothelial cells, synovial lining cells, chondrocytes, and hepatocytes (16, 17). The 68 kDa transmembrane type II receptor (IL-1 RII) is expressed on B cells, neutrophils, and bone marrow cells (18). The two IL-1 receptor types show approximately 28% homology in their extracellular domains but differ significantly in that the type II receptor has a cytoplasmic domain of only 29 amino acids (aa), whereas the type I receptor has a 213 aa cytoplasmic domain. IL-1 RII does not appear to signal in response to IL-1 and may function as a decoy receptor that attenuates IL-1 function (19). The IL-1 receptor accessory protein (IL-1 RAcP) associates with IL-1 RI and is required for IL-1 RI signal transduction (20). IL-1ra is a secreted molecule that functions as a competitive inhibitor of IL-1 (21, 22). Soluble forms of both IL-1 RI and IL-1 RII have been detected in human plasma, synovial fluids, and the conditioned media of several human cell lines (23, 24). In addition, IL-1 binding proteins that resemble soluble IL-1 RII are encoded by vaccinia and cowpox viruses (25).

The QuantiGlo Human IL-1β Chemiluminescent Immunoassay is a 5.5 hour solid phase ELISA designed to measure human IL-1β in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human IL-1β and antibodies raised against the recombinant protein. It has been shown to accurately quantitate the recombinant factor. Results obtained using natural IL-1β showed linear curves that were parallel to the standard curves obtained using the QuantiGlo kit standards. These results indicate that this kit can be used to determine relative mass values for natural IL-1β. Reports indicate that this and other ELISA kits calibrated using mature IL-1β as a standard will detect, but considerably underestimate, the unprocessed IL-1β precursor present in samples (26, 27). In biological samples other than cell lysates, the precursor form of IL-1β is usually not the predominant form of IL-1β present and, additionally, is not biologically active. Therefore, results obtained using this kit should provide a useful measure of the levels of active IL-1β present in biological fluids.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-1β has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-1β present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-1β is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, an enhanced luminol/peroxide substrate solution is added to the wells and light is produced in proportion to the amount of IL-1β bound in the initial step. A microplate luminometer is used to measure the intensity of the light emitted.

TECHNICAL HINTS

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent and repeat the assay. If cell culture supernate samples require large dilutions, perform an intermediate dilution with culture media and the final dilution with Calibrator Diluent.
- Variation in pipetting technique, washing technique, luminometers, incubation time or temperature can cause variations in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the QuantiGlo Immunoassay, the possibility of interference cannot be excluded.
- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Relative light units (RLUs) may differ among luminometers. The QuantiGlo IL-1β Immunoassay was optimized using a DYNEX TECHNOLOGIES MLX™ luminometer. Other instruments may require settings to be adjusted.
- Relative light units may vary within the 15 minute reading window.
- Due to limitations of many luminometers, it is recommended that the standards be assayed in duplicate from high to low beginning with the high standard in wells A_1 and A_2.

MLX is a trademark of DYNEX TECHNOLOGIES

MATERIALS PROVIDED

IL-1β Microplate* (Part 893171) - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against IL-1β.

IL-1β Conjugate* (Part 893172) - 21 mL of polyclonal antibody against IL-1β conjugated to horseradish peroxidase with preservatives.

IL-1β Standard* (Part 890628) - 25 ng of recombinant human IL-1β in a buffered protein base with preservatives; lyophilized.

Assay Diluent RD1W (Part 895117) - 11 mL of a buffered protein base with preservatives.

Calibrator Diluent RD6-10 (Part 895468) - 21 mL of a buffered protein base with preservatives.

Wash Buffer Concentrate (Part 895222) - 100 mL of a 10-fold concentrated solution of buffered surfactant with preservatives.

Glo Reagent A (Part 895868) - 4 mL of stabilized enhanced luminol.

Glo Reagent B (Part 895869) - 8 mL of stabilized hydrogen peroxide.

Plate Covers - 4 adhesive strips.

*This product is covered by one or more of the following U.S. patents: 4,766,069; 5,510,462; 5,681,933; 5,474,899; 5,789,185; 5,484,887.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8° C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="8">Opened/ Reconstituted Reagents</td><td>Diluted Wash Buffer</td><td rowspan="7">May be stored for up to 1 month at 2-8° C.**</td></tr>
<tr><td>Calibrator Diluent RD6-10</td></tr>
<tr><td>Assay Diluent RD1W</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>Glo Reagent A</td></tr>
<tr><td>Glo Reagent B</td></tr>
<tr><td>Standard</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8° C.**</td></tr>
</table>

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- DYNEX TECHNOLOGIES MLX luminometer set with the following parameters: 1.0 minute lag time; 0.5 sec/well read time; summation mode; auto gain on, or the equivalent.
- Pipettes and pipette tips.
- 1 liter graduated cylinder.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Human IL-1β Controls (optional; available from R&D Systems).

PRECAUTION

IL-1β is detectable in saliva and sweat. Take precautionary measures to prevent contamination of the kit reagents while running the assay.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Cell culture supernate samples may require dilution to read on the standard curve. If samples are suspected to be > 1000 pg/mL, run both undiluted and diluted in Calibrator Diluent RD6-10. If samples require large dilutions, perform an intermediate dilution with culture media and the final dilution with Calibrator Diluent.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

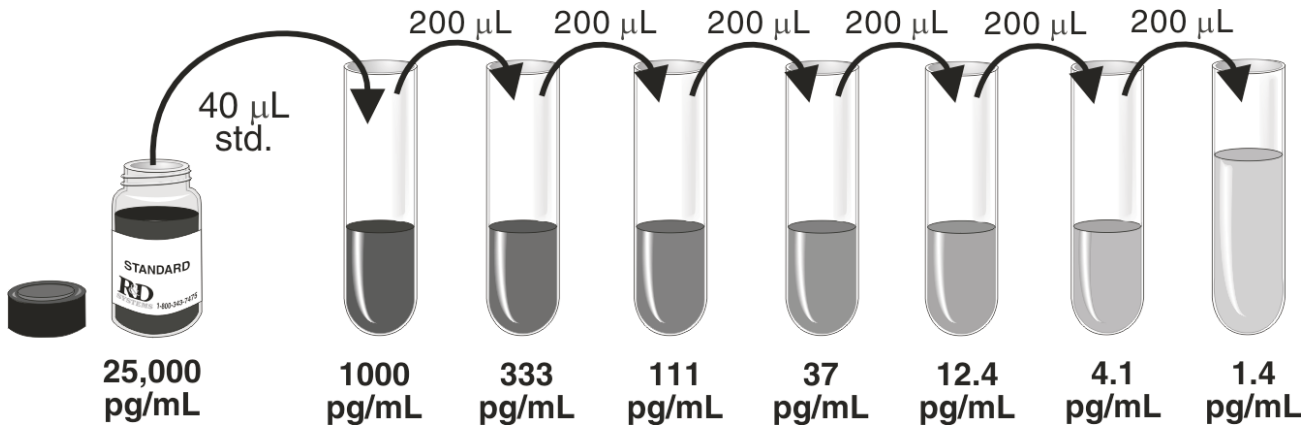
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 100 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 1000 mL of Wash Buffer.

Working Glo Reagent - 1 part Glo Reagent A (4 mL) and 2 parts Glo Reagent B (8 mL) should be mixed together 15 minutes to 4 hours before use in a plastic container with a cap. Protect from light. 100 µL of the resultant mixture is required per well.

Note: *If running the assay in less than 96 wells, mix appropriate amounts of Glo Reagent A and Glo Reagent B. To assay half a plate (48 wells), mix 2 mL of Glo Reagent A with 4 mL of Glo Reagent B. Working Glo Reagent should be discarded after use.*

Standard - Reconstitute the Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 25,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 960 µL of Calibrator Diluent RD6-10 into the 1000 pg/mL tube. Pipette 400 µL of Calibrator Diluent RD6-10 into the remaining tubes. Use the stock solution to produce a 3-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 1,000 pg/mL standard serves as the high standard. The Calibrator Diluent RD6-10 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

Note: *IL-1β is detectable in saliva and sweat. Take precautionary measures to prevent contamination of the kit reagents while running the assay.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.

2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

3. Add 100 µL of Assay Diluent RD1W to each well.

4. Add 100 µL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.

5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6. Add 200 µL of IL-1β Conjugate to each well. Cover with a new adhesive strip. Incubate for 3 hours at room temperature on the shaker.

   Note: *Prepare Working Glo Reagent at this time.*

7. Repeat the aspiration/wash as in step 5.

8. Add 100 µL of Working Glo Reagent to each well. Incubate for 5-20 minutes at room temperature **on the benchtop. Protect from light.**

9. Determine the RLU of each well using a luminometer set with the following parameters: 1.0 min. lag time; 0.5 sec/well read time; summation mode; auto gain on.

*Cell culture supernate samples may require dilution. See Sample Preparation section.

ASSAY PROCEDURE SUMMARY

1. Prepare reagents, samples, and standards as instructed.

2. Add 100 µL Assay Diluent to each well.

3. Add 100 µL Standard, sample*, or control to each well. Incubate for 2 hours on the shaker at RT.

4. Aspirate and wash each well 4 times.

5. Add 200 µL Conjugate to each well. Incubate for 3 hours on the shaker at RT.

Note: *Prepare Working Glo Reagent at this time.*

6. Aspirate and wash each well 4 times.

7. Add 100 µL Working Glo Reagent to each well. Incubate for 5 - 20 minutes **on the benchtop. Protect from light.**

8. Determine the RLU of each well using a luminometer.

*Cell culture supernate samples may require dilution.

CALCULATION OF RESULTS

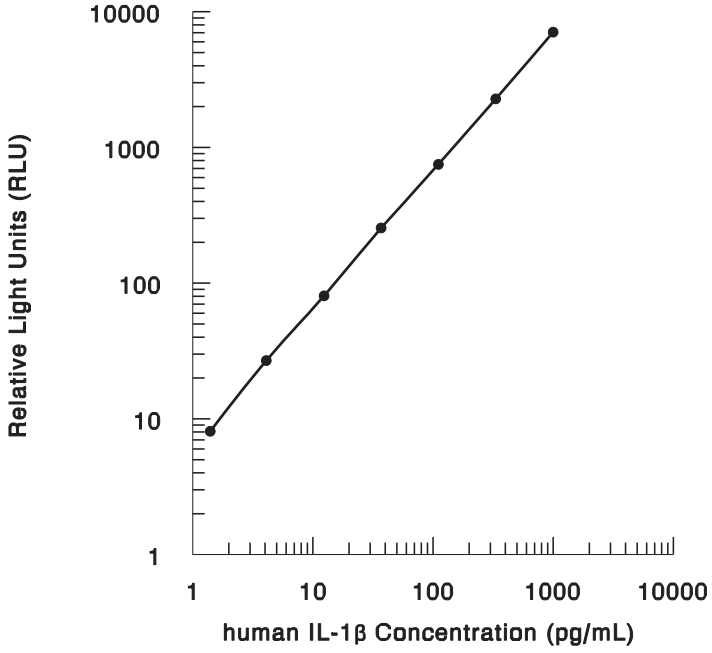
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard RLU.

Create a standard curve by reducing the data using computer software capable of generating a cubic-spline curve fit.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve was generated using a DYNEX TECHNOLOGIES MLX luminometer and is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



pg/mL	RLU	Average	Corrected
0	12.2 12.7	12.4	—
1.4	19.4 21.6	20.5	8.1
4.1	37.0 41.6	39.3	26.9
12.4	91 94	93	80.6
37	261 273	267	255
111	756 770	763	751
333	2292 2298	2295	2283
1000	7085 7085	7085	7073

PRECISION

Intra-assay Precision (Precision within an assay) Four samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Four samples of known concentration were tested in twenty separate assays to assess inter-assay precision.

	Intra-Assay Precision			
Sample	1	2	3	4
n	20	20	20	20
Mean (pg/mL)	7.96	103	638	4.18
Standard Deviation	0.31	5.0	30	0.16
CV (%)	3.9	4.9	4.7	3.8

	Inter-Assay Precision			
Sample	1	2	3	4
n	20	20	20	20
Mean (pg/mL)	9.86	136	695	5.13
Standard Deviation	0.71	9.0	38	0.40
CV (%)	7.2	6.6	5.5	7.8

RECOVERY

The recovery of recombinant human IL-1β spiked to three different levels in samples throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n = 4)	106	100-115%
Serum (n = 4)	96	87-102%
EDTA plasma (n = 4)	94	88-107%
Heparin plasma (n = 4)	93	87-103%
Citrate plasma (n = 4)	95	90-101%

LINEARITY

To assess the linearity of the assay, four samples containing or spiked with high concentrations of IL-1β in various matrices were diluted with the Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n = 4)	Serum (n = 4)	EDTA plasma (n = 4)	Heparin plasma (n = 4)	Citrate plasma (n = 4)
1:2	Average % of Expected	98	104	101	101	107
	Range (%)	94-105	102-109	99-104	98-105	103-111
1:4	Average % of Expected	96	105	96	101	111
	Range (%)	92-100	102-108	88-104	95-108	105-116
1:8	Average % of Expected	91	97	95	99	99
	Range (%)	87-96	95-101	87-98	90-106	97-104
1:16	Average % of Expected	97	102	100	103	102
	Range (%)	94-102	97-106	93-104	92-111	100-106
1:32	Average % of Expected	99	102	104	107	104
	Range (%)	92-107	97-107	97-108	95-113	101-107

SENSITIVITY

Seventy-four assays were evaluated and the minimum detectable dose (MDD) of IL-1β ranged from 0.05-0.55 pg/mL. The mean MDD was 0.16 pg/mL.

The MDD was determined by adding two standard deviations to the mean RLU of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human IL-1β produced at R&D Systems.

The NIBSC/WHO IL-1β 1st International Standard 86/680, which was intended as a potency standard, was evaluated in this kit. This standard is an *E. coli*-expressed recombinant human IL-1β.

The dose response curve of the 1st International Standard parallels the QuantiGlo standard curve. To convert sample values obtained with the QuantiGlo Human IL-1β kit to approximate NIBSC 86/680 values, use the equation below.

NIBSC (86/680) approximate value (IU/mL) = 0.0774 x QuantiGlo Human IL-1β value (pg/mL).

SAMPLE VALUES

Serum/Plasma - Thirty-five serum, EDTA plasma, and heparin plasma sample sets and twenty-one citrate plasma samples drawn from apparently healthy volunteers were evaluated for the presence of IL-1β in this assay. One serum sample and one EDTA and heparin plasma matched donor sample measured 1.93 pg/mL, 1.99 pg/mL, and 1.99 pg/mL, respectively. All other serum and plasma samples measured below the lowest IL-1β Standard. No medical histories were available for the donors used in this study.

Cell Culture Supernates - Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 50 μM β-mercaptoethanol, and 2 mM L-glutamine. Cells were cultured unstimulated or stimulated with 10 μg/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of natural IL-1β.

Condition	Day 1 (pg/mL)	Day 6 (pg/mL)
Unstimulated	13.4	3.77
Stimulated	8120	4940

SPECIFICITY

This assay recognizes natural and recombinant human IL-1β. This assay also recognizes natural and recombinant rhesus macaque IL-1β.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD6-10 and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rhIL-1β control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:	**Recombinant mouse:**	**Recombinant porcine:**
IL-1α	IL-1α	IL-1β
IL-1ra		
IL-1 sRI		
IL-1 sRII		

This assay exhibits < 1% cross-reactivity with cotton rat and rat IL-1β and < 0.2% cross-reactivity with mouse and feline IL-1β.

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PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.